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Escape behaviour of aposematic (*Oophaga pumilio*) and cryptic (*Craugastor* sp.) frogs in response to simulated predator approach

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Abstract: Crypsis and aposematism are common antipredator strategies that can each be coupled with behaviours that maximize predator deterrence or avoidance. Cryptic animals employ camouflage to conceal themselves within their environment and generally rely on immobility to avoid detection by predators. Alternatively, aposematic animals tend to rely on an association between conspicuous colouration and secondary defence to deter potential predators, and tend to exhibit slow movements in response to predators. The goal of the present study was to determine how cryptic *Craugastor* sp. and aposematic *Oophaga pumilio* respond to simulated human and bird model predators. *Oophaga pumilio* responded more often with movement to both the human (17/22) and bird (9/25) predators than *Craugastor* sp. (human: 2/21; bird: 0/21). The increased movement resulted in a greater average flight initiation distance, latency to move, and distance fled in *O. pumilio*. These findings suggest that cryptic *Craugastor* sp. rely on immobility to avoid detection, whereas aposematic *O. pumilio* utilize movement, possibly as a mechanism to increase the visibility of their warning signals to potential predators. Furthermore, *O. pumilio* exhibited greater movement in response to humans, suggesting that they actively avoid trampling by large threats, rather than considering them predators.

Key Words: anti-predator behaviour, aposematism, crypsis, dendrobatid, flight initiation distance

Most organisms are subject to strong selective pressures from predators, and prey employ a combination of morphological and behavioural adaptations to avoid predation (Blanchette & Saporito 2016, David *et al.* 2014, Toledo *et al.* 2011). Many predators are sensitive to movement, and therefore it can be advantageous for prey to remain immobile (Bulbert *et al.* 2015, Cooper *et al.* 2009a, Miyatake *et al.* 2007, Ozel & Stynoski 2011, Paluh *et al.* 2014). Crypsis is one strategy in which prey use camouflage and immobility to avoid detection; however, aposematic organisms often exhibit slow movements (Cooper *et al.* 2009a, b: Ozel & Stynoski 2011), which may act to enhance the visibility of their warning signal to predators (Ruxton *et al.* 2004).

Poison frogs in the family Dendrobatidae are aposematically coloured and chemically defended (Saporito *et al.* 2007, 2012). Dendrobatids exhibit complex social behaviours and forage diurnally in the leaf litter, which likely increases their conspicuousness and risk to potential predators, such as birds. Although many social behaviours are well described (Meuche *et al.* 2011, Savage 2002), little is known about the behavioural responses of dendrobatids to predators. Escape behaviour of anurans in response to predators has been largely quantified in the literature by measures such as latency to move, angle of escape and distance fled (Bulbert *et al.* 2015, Cooper *et al.* 2009a, b); however, flight initiation distance (the distance between predator and prey when the prey flees; FID) is the most commonly measured parameter. FID has been used to compare antipredator behaviour between cryptic and aposematic anurans, wherein humans are commonly used as a simulated predator (Cooper *et al.* 2008, Dugas *et al.* 2015, Ozel & Stynoski 2011; however, see Willink *et al.* 2013). The dendrobatid frogs *Dendrobates auratus* and *Oophaga pumilio* hop short distances in response to an approaching human (Cooper *et al.* 2008, 2009a, b; Cooper & Blumstein 2016, Dugas *et al.* 2015, Ozel & Stynoski 2011, Pröhl & Ostrowski 2011), whereas the dendrobatid *O. granulifera* varies in the degree of movement in response to a simulated bird predator, which is correlated with its

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dorsal colouration (Willink *et al.* 2013). A comparative study focused on measuring the escape responses of dendrobatid frogs to a simulated human and bird predator would provide valuable information on how frogs perceive various approaching threats.

The goal of the present study was to compare the behaviours of the dendrobatid frog *O. pumilio* to an approaching human and bird predator, by examining escape response variables (flight initiation distance, latency to move, angle of escape and distance fled) between these two simulated predator threats. Furthermore, as a comparison of the antipredator strategies between cryptic and aposematic organisms, these same escape variables were also measured in cryptic frogs in the genus *Craugastor* sp.

The present study was conducted in lowland tropical forest at the La Selva Biological Station (10°26′N, 83°59′W) in north-eastern Costa Rica from 26 February–7 March 2016. Forty-seven adult *O. pumilio* (SVL ≥ 19 mm) and 31 *Craugastor* sp. (SVL 15–25 mm) were captured during daylight hours (08h00–11h00) (Savage 2002). Upon capture, the sex of *O. pumilio* was determined based on the presence of a darkened throat patch in males (Savage 2002). *Craugastor* sp. exhibits no obvious secondary sexual characteristics (Savage 2002). All frogs were measured for SVL to the nearest 0.01 mm using Traceable® Digital Calipers and to the nearest 0.01 g for mass using a Pesola PPS200 digital pocket balance prior to behavioural assays. Individuals were housed in Ziploc bags (26.7 × 22.9 × 13.2 cm) with small amounts of leaf litter for up to 48 h post capture before being released at their original capture site. To avoid resampling of the same individuals, frogs were not collected from the same location following their release.

All behavioural assays were conducted at the Huertos Plots (STR 1200) during daylight hours (08h00–17h00) and under similar weather conditions (clear to partly cloudy). Each frog was placed onto the centre of a black plastic base (30.5 × 30.5 cm), and acclimatized under a dark cover object (8 × 7.8 × 5.2 cm) for 5 min. A researcher standing 1.5 m away lifted the cover object, and the frog was given 10 s to adjust before the simulated predator began approach from 5 m away. The simulated predator approached from a 0/360° angle relative to the position of the frog, and the angle of frog escape was based on this approach (Figure 1). The distance fled was measured as the distance the frog moved to until it either remained motionless for 5 s or passed 1.5 m. Latency to movement (time until frog moved in response to the predator) was recorded from when the simulated predator began its approach. If the frog did not move, FID, latency and distance fled were recorded as zero and the final angle at which the frog was facing was recorded.

For the bird predation treatment, 25 *O. pumilio* (14 female, 11 male) and 21 *Craugastor* sp. were used. The rufous motmot (*Baryphthengus martii*) is documented as a predator of dendrobatids (Alvarado *et al.* 2013), and therefore a life-sized model of this bird was constructed using Floracraft Floral Foam sealed with Mod Podge Sealer and painted with FolkArt® multisurface acrylic paint. A clear nylon monofilament line (3.6 kg test strength) was tied at one end to a PVC pipe at 2 m in height and on the other end to a stake in the ground 7.5 m away. The model bird was attached to this line, and once released it glided silently over each frog (average height: 0.5 m; approximate velocity: 0.9 m s^{-1}). For the human approach treatment, 22 *O. pumilio* (11 female, 11 male) and 21 *Craugastor* sp. (11 of which were also used in the bird treatment, due to low capture rates) were used. One researcher approached each frog from the same starting position as the bird model and walked at an approximate velocity of 0.9 m s^{-1}.

Independent-samples t-tests were used to compare the FID, latency and distance fled between *O. pumilio* and *Craugastor* sp. and between *O. pumilio* males and females among and within treatments. All statistical analyses were conducted in SPSS v. 14 for Windows.

In response to the simulated bird predator, nine out of 25 *O. pumilio* exhibited movement (three pivoted, six fled; Figure 1a), whereas zero out of 21 *Craugastor* sp. moved. There was a significant difference between *O. pumilio* and *Craugastor* sp. in response to the bird model for FID ($t_{24} = 2.13$, $P = 0.044$), latency ($t_{24} = 3.34$, $P = 0.003$), and distance fled ($t_{24} = 2.13$, $P = 0.043$). In response to the approaching human, 17 out of 22 *O. pumilio* exhibited movement (four pivoted, 13 fled; Figure 1b), whereas two out of 21 *Craugastor* sp. moved. There was a significant difference between *O. pumilio* and *Craugastor* sp. in response to the human for FID ($t_{21.9} = 3.04$, $P = 0.006$), latency ($t_{38.1} = 4.91$, $P < 0.05$) and distance fled ($t_{26.6} = 3.16$, $P = 0.004$; Table 1).

On average, female *O. pumilio* responded quicker to the approaching bird than male *O. pumilio*; however, there were no significant differences in response to the bird for FID ($t_{23} = 0.47$, $P = 0.640$), latency ($t_{23} = 0.48$, $P = 0.638$), and distance fled ($t_{23} = 0.48$, $P = 0.638$; Table 1) between sexes. On average, female *O. pumilio* responded quicker to the approaching human than male *O. pumilio*; however, there were no significant differences in response to the human for FID ($t_{20} = 0.84$, $P = 0.839$), latency ($t_{20} = 0.96$, $P = 0.350$), and distance fled ($t_{20} = -1.43$, $P = 0.168$; Table 1) between sexes.

Cryptic organisms generally rely on camouflage and immobility to reduce predation risk, while aposematic organisms rely on conspicuous colouration and some degree of movement to deter predators (Ruxton *et al.* 2004). Cryptic frogs commonly remain motionless in the presence of a simulated human predator (Cooper *et al.* 2008), whereas aposematic frogs have been found to flee, but their movement is characterized by slow hops (Cooper

Table 1. The average flight initiation distance (FID), latency, and distance fled (± SE) for *Oophaga pumilio*, *Oophaga pumilio* males and females, and *Craugastor* sp. that exhibited movement in response to approaching bird and human simulated predators at La Selva Biological Station, Costa Rica. The number fled refers to the number of individuals that attempted escape and the number pivoted refers to the individuals that remained in place but changed orientation.

Species	Predator	Average FID (cm)	Average latency (s)	Average distance fled (cm)	Number fled	Number pivoted
Oophaga pumilio	Bird	6.8 ± 3.2	1.5 ± 0.4	3.4 ± 1.6	6	3
Male	Bird	5.1 ± 5.0	1.2 ± 0.6	2.6 ± 2.6	2	1
Female	Bird	8.2 ± 3.8	1.7 ± 0.6	4.1 ± 1.7	4	2
Craugastor sp.	Bird	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0	0
Oophaga pumilio	Human	70.9 ± 21.6	2.7 ± 0.3	56.2 ± 17.6	13	4
Male	Human	66.4 ± 29.9	2.3 ± 0.4	76.3 ± 17.5	7	0
Female	Human	77.5 ± 32.7	3.0 ± 0.6	36.1 ± 22.0	6	4
Craugastor sp.	Human	4.4 ± 3.0	0.4 ± 0.3	7.7 ± 5.1	2	0

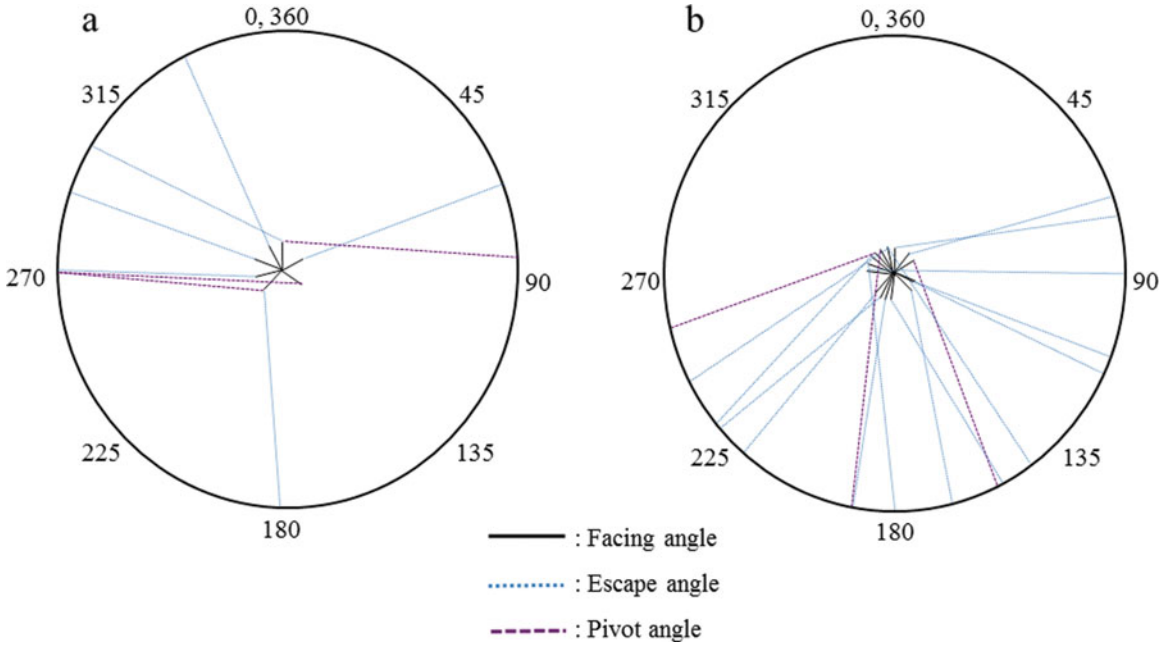

Figure 1. The facing, escape, and pivot angles of *Oophaga pumilio* that exhibited movement in response to the approaching bird (a) and the approaching human (b) at La Selva Biological Station, Costa Rica. The facing angle refers to the orientation of the individual when the cover object was lifted. The escape angle refers to the direction of individuals that fled and the pivot angle refers to individuals that changed orientation but did not flee. Both predators approached from 0/360°.

et al. 2009a, b). Our study supports previous findings that cryptic *Craugastor* sp. will not flee from an approaching threat (Cooper *et al.* 2008, 2009a). Conversely, *O. pumilio* was more responsive to the approaching human when compared with the bird predator. Most *O. pumilio* individuals fled directly away from or perpendicular to the approaching human (Figure 1b), and for longer distances, whereas fewer frogs exhibited movement in response to the model bird. Of those *O. pumilio* individuals that moved in response to the bird, their movement was not as far and appeared to be more erratic (Figure 1a). Other studies have reported that escape trajectories of anurans are typically directed away from simulated terrestrial predators (Lippolis *et al.* 2002, Royan *et al.* 2010), but are more variable in response to simulated aerial threats (Cooper *et al.* 2008).

Dendrobatids are at risk of predation by certain bird predators and there is some evidence that movement may be important to *O. pumilio*, in part due to the potential risk of birds mistaking them as fruit or seeds (Paluh *et al.* 2015). Further, movement may increase the conspicuousness of aposematic individuals to avian predators by enhancing their warning signal (Pröhl & Ostrowski 2011, Ruxton *et al.* 2004). While studies of predation upon clay model replicas have shown that birds attack some aposematic frogs, movement of model replicas significantly decreases avian predation (Paluh *et al.* 2014, 2015, Willink *et al.* 2013). Counter to the prediction that frogs would flee, many *O. pumilio* individuals remained immobile or moved slightly when approached by the bird model, suggesting that conspicuous warning

colouration, associated with some movement, is an effective defence.

The majority of escape-behaviour studies conducted with anurans have used humans as an approaching threat. In the current study, most *O. pumilio* individuals exposed to the approaching human had greater fleeing distances than those exposed to the bird model (Table 1). The increased fleeing distance in response to a human is similar to previous studies (Cooper *et al.* 2009a, b; Ozel & Stynoski 2011), and may suggest that frogs view humans as a risk of trampling and not predation. Humans are large objects and produce vibrations when walking, which may cause the frogs to move sooner than would be expected if an actual predator were approaching (Cooper *et al.* 2009b). The comparison between *Craugastor* sp. and *O. pumilio* highlights the difference in antipredator strategies between cryptic and aposematic organisms. The likelihood of *Craugastor* sp. being detected is low if it remains immobile in response to a human or bird; however, some movement of *O. pumilio*, particularly in response to a bird, may increase the visibility of its aposematic signal to a perceived threat.

The current study suggests that male and female *O. pumilio* do not exhibit any significant differences in escape behaviour in response to an approaching bird or human; however, females were more likely to initiate escape sooner, with respect to FID, in response to both simulated predators (Table 1). Males may experience greater pressure to remain stationary in response to a predator because of the energy that would be required to return to their territories and the potential for missed mating opportunities (Dugas *et al.* 2015).

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